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Short-term effect of a high-protein/low-carbohydrate diet on aminopeptidase in adult rat jejunoileum

Site of aminopeptidase response

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The short-term effects of high-protein/low-carbohydrate diet on aminopeptidase N activity were studied in the brush-border membranes of proximal jejunum and proximal ileum of adult rats. The animals were starved overnight and re-fed for 15 h either with a standard diet (20% protein, 55% carbohydrate, in terms of energy content) or with a high-protein/low-carbohydrate diet of equal energy content (70% protein, 5% carbohydrate). All rats consumed similar amounts of diet, and measurements were made 15 h after initiation of re-feeding. In the proximal jejunum a slight increase in aminopeptidase activity was observed after the high-protein intake. In contrast, considerable stimulation (52%) of the enzyme specific activity was obtained in the proximal ileum. This increase in ileal aminopeptidase activity was more prominent in the mature cells of the upper villus. To determine if the increase of aminopeptidase activity was due to an increased amount of enzyme protein, rocket immunoelectrophoresis was performed with detergent-solubilized brush-border protein from ileum on agarose gels containing anti-(rat brush-border) antiserum. When the same amount of enzyme activity was loaded on the gels, the peaks of immunoprecipitate for aminopeptidase were similar for animals fed on a standard or a high-protein diet. When the same amount of protein was loaded, the peak of immunoprecipitate for aminopeptidase was higher (81%) after a high-protein diet. These results showed that the high protein intake evoked an increase in aminopeptidase activity, with a concomitant increase in the amount of immunoreactive protein.

INTRODUCTION

Aminopeptidase N (EC 3.4.11.2) is located in the brush-border membrane of enterocytes and plays an important role in the final stages of protein digestion (Ugolev & Laey, 1973; Adibi & Kim, 1981). This enzyme hydrolyses short oligopeptides which are present in the intestinal lumen, and the amino acids formed are directly transferred to the specific membrane transporters (Vorotyntseva *et al.*, 1984). Despite the well-documented characterization of the physicochemical and enzymic properties of brush-border aminopeptidases in several species (Maroux *et al.*, 1973; Kim & Brophy, 1976; Gray & Santiago, 1977; Kocna *et al.*, 1980; Norén & Sjöström, 1980; Feracci *et al.*, 1982; Ahnen *et al.*, 1983; Tobey *et al.*, 1985), the mechanisms involved in the regulation of these hydrolases remain largely unknown. Several results have shown that the activity of aminopeptidase can be increased after feeding rats for several days or weeks with a high-protein diet (Nicholson *et al.*, 1974; Saito & Suda, 1975; Kimura *et al.*, 1977; McCarthy *et al.*, 1980; Gaucher & Puigserver, 1985). An abrupt increase in aminopeptidase synthesis was observed after a 30 min intraluminal perfusion of a tetrapeptide or a synthetic substrate in jejunum of rats (Reisenauer & Gray, 1985).

To gain more insight into the adaptation of intestinal aminopeptidase after dietary changes, we decided to determine the short-term response of aminopeptidase activity in the jejunoileum of adult rats 15 h after feeding with a high-protein diet. The aim of our study was to define the site of early response of aminopeptidase along the length of the small intestine and the villus–crypt axis, and to investigate whether the changes in aminopeptidase activity were related to modifications in the amount of aminopeptidase N protein or to activation of pre-existing inactive enzyme molecules.

METHODS

Animals and diets

Male Wistar rats (body wt. 290–320 g) were housed individually in metabolic cages, and food was removed at 17:00 h, but free access to water was allowed. The next day at 17:00 h the animals were given either a standard diet containing (in terms of energy) 20% protein and 55% carbohydrate or a high-protein/low-carbohydrate diet of equal energy content, containing 70% protein and 5% carbohydrate. Both diets were provided by the same manufacturer (UAR, Villemoisson sur Orge, France) and contained qualitatively and quantitatively the same amount of fat (4%), minerals (5%) and vitamins. The carbohydrate source and the protein source were respectively starch and casein. The allowance of each diet was 20 g per rat. Animals which consumed less than 18 g during the night were eliminated from the study. The animals were then killed the next morning at 08:00 h (15 h after the initiation of feeding). The proximal parts of the jejunum and the ileum were resected. The proximal part of the jejunum comprised the first 10 cm extending from the ligament of Treitz. The proximal part of the ileum consisted of a 10 cm segment extending from the middle of the jejunoileum.

Enzyme assays

The intestinal segments were everted, and the mucosa was scraped and homogenized in ice-cold 50 mM-mannitol/2 mM-Tris/HCl buffer (pH 7.4). Brush-border membranes were purified as described by Schmitz *et al.* (1973). Aminopeptidase activity was assayed with L-alanine *p*-nitroanilide as substrate (Maroux *et al.*, 1973). Enzyme activities were expressed as either specific activities (munits/mg of protein) or as total activities (munits/ segment). One unit of activity equals 1 μmol of product formed/min at 37 °C. Protein was assayed by the method of Lowry *et al.* (1951).

Cell separation

The intestinal segments were flushed with 0.9% NaCl and everted. To obtain a sequential cell release from villus tip to crypt base, the everted segments were submitted to successive incubations in dissociating medium under agitation in a water bath (Raul *et al.*, 1977). After incubation for 10 min the cells were collected by centrifugation (800 *g*). A portion of each cell fraction was removed for determination of enzyme activities. The sequentially isolated cells were pooled into two fractions. The upper-villus fraction included the first 40% of isolated cells, and was enriched in mature epithelial cells. The crypt fraction included the last 30% of the isolated cells formed by the immature epithelial cells of the villus and crypt base (Raul & Von der Decken, 1985).

Rocket immunoelectrophoresis

Brush-border antigen was prepared as described by Skovbjerg *et al.* (1978). The brush-border membrane pellet was suspended in 50 mM-Tris/HCl buffer (pH 8.0) containing 1% Triton X-100 and incubated for 1 h at 4 °C with frequent stirring. The insoluble material was removed by centrifugation at 20000 *g* for 30 min; the resulting supernatant contained the solubilized brush-border protein. The solubilized proteins were used for immunization of rabbits by the scheme described by Triadou *et al.* (1983). The specificity of the antisera was assessed on histological sections of rat intestinal mucosa by a double-layer immunofluorescence technique (Skovbjerg *et al.*, 1978). Rocket immunoelectrophoresis (Laurell, 1972) was performed on glass plates layered with 1% agarose (Sigma Chemical Co., St. Louis, MO, U.S.A.) in 30 mM-Tris/barbital buffer (pH 8.8)/0.5% Triton X-100. A 50-fold dilution of the antiserum was used in the gel, and either 15 μg of protein or 30 munits of enzyme activity was applied to the wells. Electrophoresis was run for 17 h at 12 V/cm. After electrophoresis, the glass plate was washed with 0.9% NaCl, pressed with filter paper for 15 min and dried. The gels were either stained for protein with Coomassie Brilliant Blue or stained for peptidase activities. The following reaction mixture was used to identify aminopeptidase activity: 10 mg of L-alanine β-naphthylamide together with 20 mg of Fast Garnet GBC salt (Sigma) in 15 ml of 50 mM-Tris/HCl buffer, pH 8.0. Aminopeptidase appeared as red bands.

Statistical analysis

Statistical analysis was performed with one-way analysis of variance followed by Student's *t* test. A P value < 0.02 was considered as indicating a significant difference between two mean values.

RESULTS

Protein content and aminopeptidase N activity in the proximal jejunum and ileum

The body weights of the rats were not modified 15 h after the initiation of feeding as compared with the initial weight. The initial (pre-starvation) body weight of rats was 317 ± 10.7 g (24) (mean ± S.E.M.; *n*); weight at time of death for the standard-diet group was 310 ± 9.1 g (12), and 290 ± 6.8 g (12) for the high-protein-diet group. There were no diet-dependent differences observed in the protein content of the jejunal or ileal segments (Table 1). The effects of the high-protein diet on aminopeptidase activity in the jejunal and ileal segments were already maximum 15 h after re-feeding with the diet and did not vary significantly over a 63 h period (Table 1). The protein contents of the brush-border membranes from jejunal and ileal segments were similar for both dietary groups (Table 1). The yield of brush-border protein was significantly higher in the ileal segment as compared with

Table 1. Effects of a high-protein diet on aminopeptidase N activity in the proximal jejunum and ileum

The results are means ± S.E.M. ($n = 12$). Rats were starved for 24 h and re-fed either with a standard diet or with a high-protein diet of equal energy content for 15 h: * $P < 0.01$ between standard and high-protein diet; † $P < 0.01$ between jejunum and ileum for a given diet. Values of refed animals for 39 and 36 hours did not significantly differ from these values [jejunum 427 ± 35 and 497 ± 40 respectively; ileum 724 ± 5.6 and 767 ± 58 respectively (means ± S.E.M.; $n = 6$).

		Mucosal homogenates		Brush-border membranes			
Tissue	Diet	Protein content (mg/segment)	Enzyme activity (munits/mg of protein)	Protein content (mg/segment)	Yield (%)	Enzyme activity (munits/mg of protein)	Total activity per segment (munits)
Jejunum	Standard	76.7 ± 3.8	30.3 ± 2.2	3.4 ± 0.2	4.3 ± 0.2	340 ± 27.4	2253 ± 106
	High-protein	86.2 ± 5.0	35.0 ± 1.6	3.5 ± 0.2	3.5 ± 0.1*	423 ± 22.8	2987 ± 134*
Ileum	Standard	74.8 ± 4.8	49.5 ± 2.7†	4.0 ± 0.2	5.6 ± 0.4†	459 ± 25.5†	3566 ± 198†
	High-protein	73.9 ± 4.0	71.0 ± 2.3*†	4.2 ± 0.3	5.7 ± 0.2†	697 ± 38.5*†	5523 ± 144*†

the proximal jejunum ($P < 0.01$). No diet-related differences were observed in the brush-border-protein yield in the ileum, whereas a lower yield ($P < 0.01$) was obtained in the jejunum after the ingestion of the high-protein diet.

Independently of the diet, the activity of aminopeptidase was significantly higher in the ileal segments as compared with the proximal jejunum (Table 1). As shown in Table 1, the dietary-induced modification of aminopeptidase activities were not identical in both intestinal segments. The high-protein diet led to no significant changes in the mucosal specific activity, but to a slight increase in the total activity of aminopeptidase in the jejunal segment and isolated brush-border membranes. Substantial changes occurred in the ileal segment, where the high-protein diet induced a significant stimulation (43–55%) of specific and total activities of aminopeptidase in the mucosa and in the brush-border membranes (Table 1).

Effect of diet on the amount of immunoreactive aminopeptidase N

To determine if the higher aminopeptidase activity in the animals fed on a high-protein diet is related to the increase in enzyme amount, detergent-solubilized brush-border-membrane proteins obtained from the proximal ileum were quantified by rocket immunoelectrophoresis (Fig. 1). Aminopeptidase activity in the gel was detected by specific staining. When the same amount of enzyme activity was applied to the well, the height of the immunoprecipitates obtained for aminopeptidase N showed no significant modifications related to the diet. However, when the same amount of protein was applied to the well, the height of the peak for aminopeptidase N was significantly higher in rats receiving the high-protein diet (10.5 ± 0.5 mm) as compared with the controls (5.8 ± 0.3 mm; $P < 0.001$).

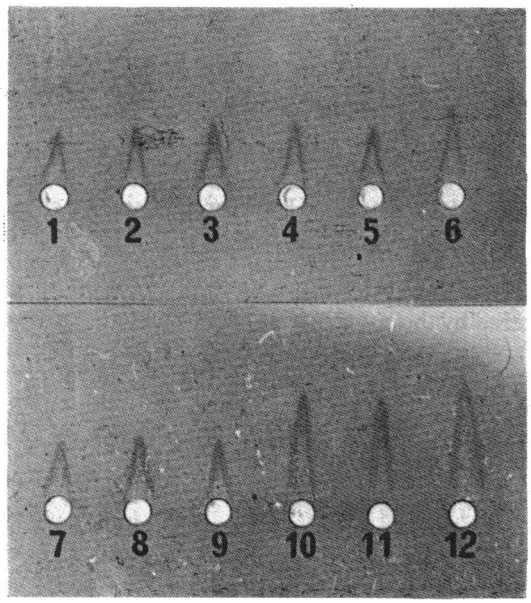

Fig. 1. Rocket immunoelectrophoresis of Triton X-100-released brush-border proteins from ileal mucosa against the corresponding antisera

The gel after electrophoresis was stained specifically for aminopeptidase N activity. Samples obtained from six different animals were applied in the wells. The samples applied to wells 1, 2, 3 (standard diet) and 4, 5, 6 (high-protein diet) contained the same amount of enzyme activity (30 munits). The samples applied to wells 7, 8, 9 (standard diet) and 10, 11, 12 (high-protein diet) contained the same amount of protein (15 μg).

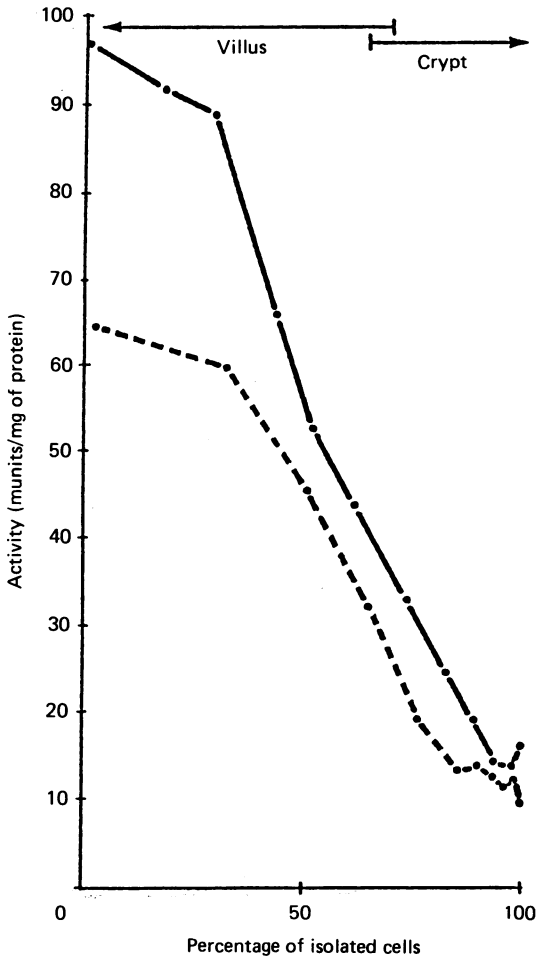

Fig. 2. Representative gradient of aminopeptidase activity in intestinal cells isolated along the villus–crypt axis in rats re-fed with a standard diet (----) or with a high-protein diet (——)

100% value for cells isolated corresponds to the sum of fractions, expressed as protein. The percentage of cells isolated from a given fraction includes the sum of the preceding fractions

Site of aminopeptidase N stimulation along the villus–crypt axis

The activity of aminopeptidase in cells sequentially isolated along the villus–crypt axis of the ileal segments was determined; Fig. 2 shows a representative pattern. The pattern obtained for aminopeptidase activity was similar for both dietary groups; however, the activity reached at the villus tip was higher in the rats fed with the high-protein diet. As illustrated in Fig. 3, after ingestion of the high-protein diet, specific aminopeptidase activity in the upper villus (mature) enterocytes was remarkably

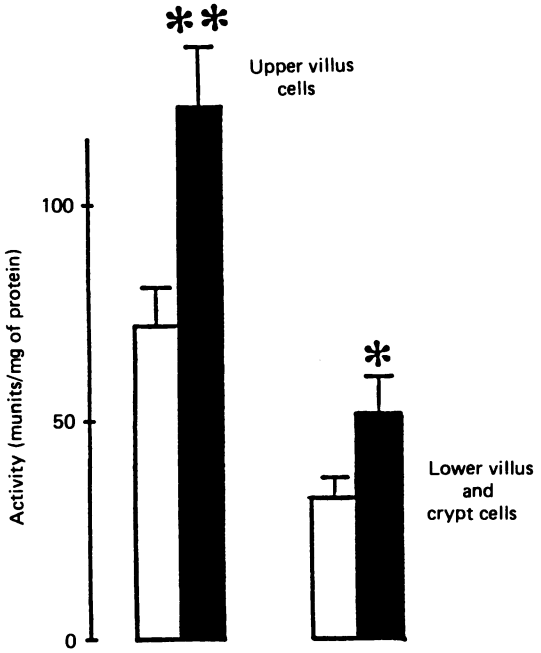

Fig. 3. Specific activities of aminopeptidase in the cells isolated from the upper villus and in those isolated from the lower villus and crypt

The upper-villus cell fraction included the first 40% of isolated cells, and the lower-villus and crypt cell fraction corresponded to the last 30% of the isolated cells. Results are means ± S.E.M. for eight rats. Significant differences from the values obtained in animals fed on standard diet are shown by $*P < 0.02$, $**P < 0.001$. □, Control diet; ■, high-protein diet.

higher (70%; $P < 0.001$) than that found in rats fed on the standard diet. A less significant difference ($P < 0.02$) in aminopeptidase activity was observed in the immature cell population obtained from the lower villus and crypt (Fig. 3).

DISCUSSION

The present study was undertaken in order to measure the early responses of brush-border hydrolases in the jejunum and ileum of adult rats after the intake of a high-protein/low-carbohydrate diet. To investigate the enzyme response to dietary changes, a period of 15 h seemed to us the most suitable, since this period is shorter than the renewal time of the cells along the villus axis (about 48 h in the adult rat), allowing us to determine the site of enzyme response along the villus–crypt axis (Fig. 3). Furthermore, our results show that the aminopeptidase activity remained stable in the brush-border membrane up to 63 h after the initiation of feeding with the high-protein diet (Table 1).

Our study confirms and extends the previous knowledge of the adaptive responses of intestinal peptidase to dietary proteins. The animals receiving a standard diet exhibited regional differences for microvillus aminopeptidase activity between both intestinal segments. The present data show that the specific and total activities of aminopeptidase are higher in the proximal ileum as compared with the proximal jejunal segment. Similar observations have been previously reported in the intestine of human and rat, showing that the ileal segments contained higher peptide hydrolase activities (McCarthy *et al.*, 1980; Triadou *et al.*, 1983). On the other hand, one report (Miura *et al.*, 1983) has shown that microvillus aminopeptidase activity was higher in the jejunum than in the ileum. This discrepancy probably arises from the fact that those authors determined enzyme activity in the distal ileum in a region near the caecum, which normally contains only low hydrolase activities.

We show that re-feeding with a high-protein diet after starvation for 24 h led to a significant increase of aminopeptidase activity in the proximal ileum within 15 h. A similar effect on the peptide hydrolase activities in the distal small intestine has been reported by others in rats fed on a high-protein diet for 7 days (McCarthy *et al.*, 1980). The existence of only a slight adaptive response for aminopeptidase activity in the proximal jejunum might be related to stepwise digestion of the food protein molecules. We speculate that food protein has first to be degraded in the lumen to smaller peptides to exert its stimulatory effect on peptidase activities. It has been reported that luminal trypsin activity is higher in the lower part of the small intestine than in the upper part (Goda & Koldovský, 1985).

Our results show that, 15 h after feeding with a high-protein diet, the increase of aminopeptidase activity in the ileum parallels the amount of corresponding aminopeptidase protein and is not related to substrate-dependent activation of pre-existing enzyme molecules. The observed increase in the number of aminopeptidase molecules may result from either an increased rate of enzyme synthesis or a decreased degradation of enzyme molecules, owing to enzyme protection by the dietary proteins, leading to an accumulation of enzyme molecules. Reisenauer & Gray (1985) have reported an abrupt induction of intestinal peptidase synthesis in jejunum after a 30 min intraluminal perfusion of a single tetrapeptide substrate. However, the site of stimulation of synthesis along the villus–crypt axis was not investigated in their study. Furthermore, the molecular mechanisms involved in the transduction of a signal triggered by the components of the diet to the intracellular machinery at a transcriptional or translational level remain still unknown. However, we have previously shown that gene expression can be modulated in the intestinal epithelial cells by diet (Raul & Von der Decken, 1985).

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